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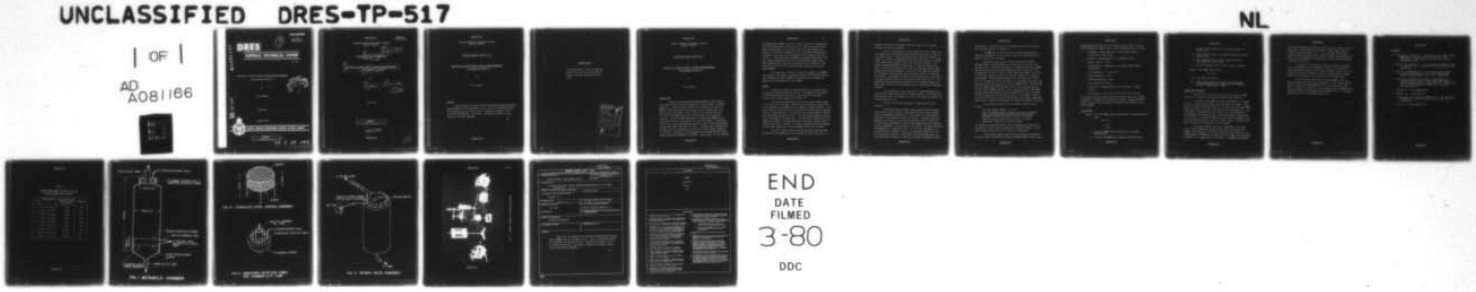

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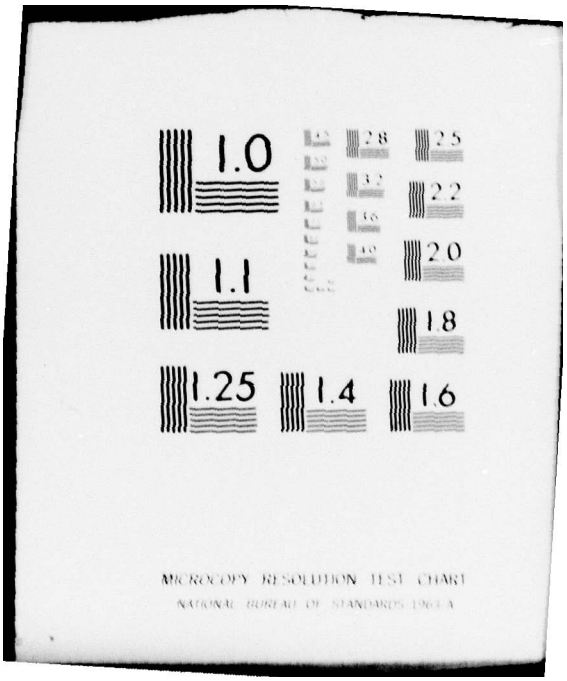
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EXCRETION OF INJECTED DIMETHYL MORPHOLINOPHOSPHORAMIDATE ON THE BREATH OF RATS (U)

by

M.L. McDonald

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ABSTRACT

Male rats were injected with undiluted dimethylmorpholinophosphoramidate (DMMPA) into the peritoneal cavity. Levels on the exhaled breath were determined over the next 48 hours. Recovery was between 1% and 2% of the administered dose. The analysis indicated the presence of metabolites of the parent compound.

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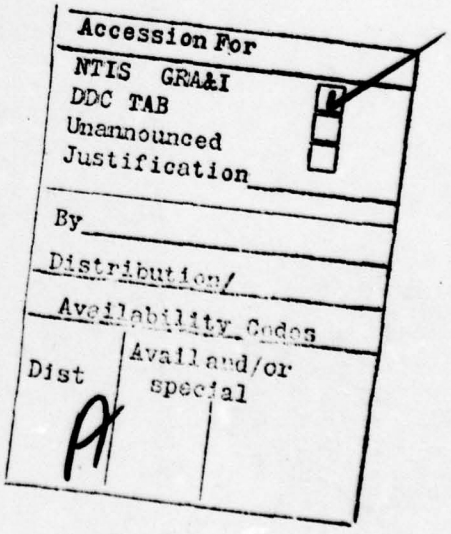

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INTRODUCTION

Dimethyl morpholinophosphoramidate (DMMPA) has been used as a nerve agent simulant to assess standard operating procedures in chemical defence (1). Some of the work carried out to establish the safety of DMMPA has been concerned with the recovery of the simulant when it was applied to man and animals. Early studies (1,2) indicated that only 25 to 50% of the DMMPA applied to the skin of man could be found in the urine. Studies by Shaw and McDonald (3) showed that DMMPA was not accumulated in any of ten different rat tissues and organs. The maximum level in the tissues and organs was reached in about 18 hours and returned to near zero values within 48 to 72 hours. A balance study indicated that the major route of excretion was the urine, with small amounts appearing in the feces. Only 50% of the injected dose was recovered and the results showed no apparent breakdown of the DMMPA. Dawson et al. (4) investigating

the persistence of DMMPA in various tissues of the rat also showed that significant amounts were found in the liver, kidneys and brain 4 hours after intraperitoneal (i.p.) injection and only trace amounts after 48 hours. They further reported that 58% of the administered dose was recovered in the urine, with the majority being excreted in the first 24 hours. It was stated that the DMMPA was probably metabolized in the body, however degradation could not be demonstrated in human plasma. Shaw and McDonald (3), based on observations made by Coleman (5), suggested that excretion on the breath probably accounted for the unrecovered DMMPA.

This study was carried out to establish whether or not DMMPA was excreted on the breath. A technique based on the miniature detector tube procedure for sampling DMMPA in the field (6) was developed to sample the exhaled breath of rats for DMMPA.

METHODS

A metabolic chamber was designed and built to provide a means of sampling the breath exhaled by the rat. The chamber was cylindrical in configuration and was made from a borosilicate glass cylinder of 100 mm ID with a total length of 37 cm (Fig. 1).

The design incorporated the use of two stainless steel screens of 3/8 inch and 1/8 inch mesh, respectively, positioned 25 mm apart (Fig. 2) and located at the bottom end of the chamber as shown in Figure 1. The upper screen, of 3/8 inch mesh, was the platform on which the rat remained during the sampling period. The urine and feces passed through to the bottom screen which retained the feces. The urine passed through to the bottom of the chamber to an outlet tube. To minimize any interruption of the chamber environment, a syringe connected to the outlet tube was used to withdraw the urine as required. It should be noted that the main purpose of the screen assembly was to insure maximum comfort for the animal.

An airflow ingress tube was located approximately 45 mm from the bottom of the chamber and 3 to 5 mm below the upper screen (Fig. 1).

A metered airflow was introduced through this tube over the rat and exited at the top of the chamber.

The air, containing the exhaled breath of the rat, flowed from the chamber via an 18 mm OD glass tube mounted in the centre of a No. 12 size rubber stopper. Twelve miniature detector tubes also fitted in the stopper in a circular configuration around the exit tube (Fig. 3) sequentially sampled the airstream prior to its exiting the chamber. The sampling procedure employed a second pump, a 12-position rotary valve with a crystal controlled electronic timer and miniature detector tubes charged with Tenax GC 60/80 mesh. The latter three items were supplied by the Chemistry Section, DRES, and are normally used for air sampling in the field. The method of connecting the miniature tubes to the rotary valve was modified to meet the requirements of the study (Fig. 4). The pump drew a metered flow of the chamber air to be sampled through a detector tube. A critical orifice monitored by a flow rate meter regulated the flow.

The activation of a tube from static to sampling mode was controlled by the rotary valve which was indexed through its 12 positions by the electronic timer. The timer was set for 1 hour sampling intervals per miniature detector tube.

Figure 5 illustrates the metabolic chamber with ancillary equipment.

Flow-rated miniature detector tubes supplied by the Chemistry Section were set up as follows. Approximately 40 mg of Tenax GC 60/80 mesh (Chromatographic Specialties Ltd.) was packed in a 38 mm length of borosilicate glass tubing of 4 mm OD and 2 mm ID. The Tenax GC bed was supported in the tube by stainless steel 200 mesh screens at each end. The tubes were conditioned for use by thermal desorption as per Chemistry Section's procedures. (The procedure is as follows: the tubes are placed in uncapped vials in quantities of 20 - 24; the vials are placed in a desiccator which is heated to 180°C in an oven and evacuated to 5×10^{-3} torr for 36 hours; the oven is then cooled, the vacuum pump turned off and the desiccator returned to atmospheric pressure by bleeding in super

pure nitrogen. Finally, the vials are capped and stored till the miniature tubes are required for sampling.)

The DMMPA was certified 99% pure as supplied from TeroChem Laboratories, Edmonton. It was stored at -66°C until received from the Chemistry Section for this study.

Male albino rats obtained from the University of Alberta (Sprague-Dawley), weighing between 190 and 250 grams, were used. Male and female rats, as reported by Coleman (5), respond identically to DMMPA. The rats were starved for 24 hours immediately prior to the run. This included control animals as well as those treated with DMMPA. At the end of the 24 hour starvation period the rats were injected (i.p.) with undiluted DMMPA. The doses used throughout the study were 0.1 mL (122.3 mg), 0.2 mL (245.6 mg) and 0.4 mL (489 mg). The weight of each dose level was calculated using a density of 1.2228 for DMMPA at 25°C. The injection site was swabbed with distilled water using a surgical gauze sponge to insure no surface DMMPA remained. The rat was then placed in the chamber and the chamber environment was sampled for DMMPA over the next 48 hours. The animal remained in the starved state while in the chamber. Control runs were carried out by placing untreated rats in the chamber immediately after 24 hours starvation and sampling the chamber environment for 48 hours.

The operating conditions for the metabolic chamber were as follows:

- flow rate through chamber = 1.0 L/min
- flow rate through miniature detector tubes while in sampling mode = 0.06 L/min average (a check made on this flow rate throughout a 48 hour run showed less than 5% variation)
- sampling time per miniature detector tube = 60 minutes
- temperature = ambient (air conditioned room)

The chamber was washed down between runs by submerging in chromic acid solution for 4 hours, then in 5% Contrad 70 solution overnight. It was then rinsed and dried at 75°C for 4 hours in a drying oven.

The miniature detector tubes (48 for a 48 hour run employing a 60 minute sampling period) were analyzed for DMMPA content via Gas Liquid

Chromatography (GLC) and the total recovery was calculated. The conditions employed for operation of the gas chromatograph were as follows:

- Instrument: Varian 1800 (data collection and processing carried out on a PDP 11/34)
- Detector: Flame photometric with phosphorus filter
- Column: Pyrex 6' x 1/8"
- Packing Support: Chromosorb W, Acid washed, DMCS treated, 80/100 mesh
- Stationary Phase: 2% Triton X305
- Column Temperature: 185°C
- Detector Temperature: 220°C
- Inlet Temperature: 230°C
- Carrier Gas: Nitrogen (Super Pure), flow rate: 35 mL/min @ 42 psi
- Inlet System: 3-way valve of local design (W.J. Fenrick)

The miniature detector tube was injected into the 3-way valve, allowed to heat for 20 seconds then flushed with nitrogen. Standardization was carried out by injecting a series of standards ranging from 3.3 ng to 6,600 ng DMMPA.

CALCULATIONS

Part I: Total mg/L DMMPA vapour concentration in miniature detector tubes:

$$S_{TV} = T_S \cdot F_1$$

$$X = \frac{X_1}{S_{TV}}$$

X = total mg/L DMMPA vapour concentration in miniature detector tubes

X_1 = the sum of the DMMPA (mg) recovered from the miniature

detector tubes (Normally this involved 48 tubes for a 48 hour run.)

S_{TV} = total volume of chamber air sampled through the miniature detector tubes

T_S = total sampling time in minutes (Normally 48 hours x 60 minutes/hour = 2880 minutes.)

F_1 = flow rate through miniature detector tubes in L/minute

Part II: Total DMMPA (mg) recovery:

$$R_T = A_{TV} \cdot X$$

R_T = total DMMPA (mg) recovery

A_{TV} = actual total volume (L) of sample stream that passed through chamber during the sampling period (Normally 1.0 L/min x 2880 minutes = 2880 L.)

RESULTS AND DISCUSSION

GLC analysis of the miniature detector tubes showed a DMMPA recovery of 1% to 2.2% of the total administered dose (Table I).

In addition to the DMMPA peak, the chromatograms of the breath from the 9 treated animals showed 2 and sometimes 3 small peaks. These were of a phosphorus bearing origin with a retention time less than DMMPA. These peaks were not present on the breath of the 4 untreated rats. The importance of this observation was the possibility that, metabolites of the parent compound were present. These peaks cannot be quantitated nor identified. It should be noted that due to their low magnitude, they would not add appreciably to the total recovery figure. Therefore, they were not taken into consideration for this purpose.

A recovery study was carried out to determine if any significant amount of DMMPA was adsorbed on the interior surfaces of the chamber, thus resulting in a low total recovery for the treated rat runs. Weighed amounts of DMMPA were introduced into the chamber and the sampling procedure for the vapour was run for 48 hours. The DMMPA was reweighed

and the miniature detector tubes were analysed and the percent recovery calculated. Several runs were carried out. These showed variable recovery, ranging from 82% to 95%. It was concluded that there was some loss due to adsorption on the interior surfaces in the chamber, but not of sufficient quantity to significantly increase the total recovery figure for the rat runs.

In answer to the original question, the study has indicated that rats injected (i.p.) with DMMPA do excrete it on the breath as well as possible metabolites of the parent compound. However, it is not excreted in sufficient quantity to explain the unaccounted for DMMPA (nearly 50%) in previous tissue, blood, feces and urine studies.

To fully determine the fate of injected DMMPA in the rat, further studies would have to be carried out. The most likely approach would be to confirm whether DMMPA is, or is not, metabolized in the body. To answer this question, a similar study to Shaw and McDonald (3) could be carried out using double-labelled DMMPA, with C^{14} in the methyl groups and tritium in the morpholine ring (8).

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TABLE I

DMMPA RECOVERY FROM THE BREATH OF THE RAT
AFTER INTRAPERITONEAL INJECTION

Run	DMMPA Injected (mg)	Total Recovery (mg) Miniature Tubes (GLC Analysis)	% Recovery
1	125.5 (0.1 mL)	1.9270	1.57
2	125.5 (0.1 mL)	1.2289	1.00
3	125.5 (0.1 mL)	1.3599	1.11
4	245.0 (0.2 mL)	2.5816	1.05
5	245.0 (0.2 mL)	2.5761	1.05
6	245.0 (0.2 mL)	3.5614	1.45
7	490.0 (0.4 mL)	10.3130	2.10
8	490.0 (0.4 mL)	5.8539	1.19
9	490.0 (0.4 mL)	10.9300	2.23

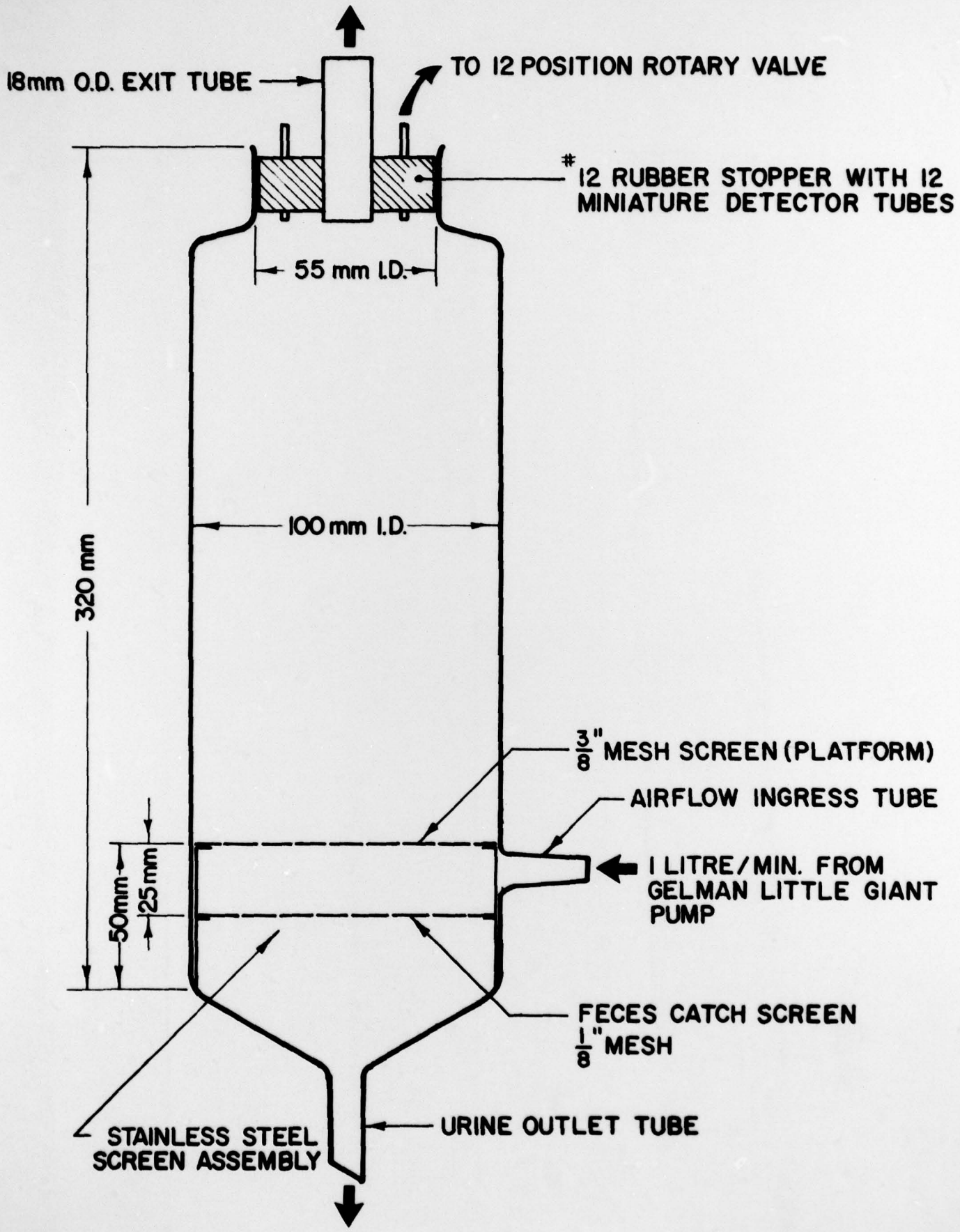

FIG. 1 METABOLIC CHAMBER

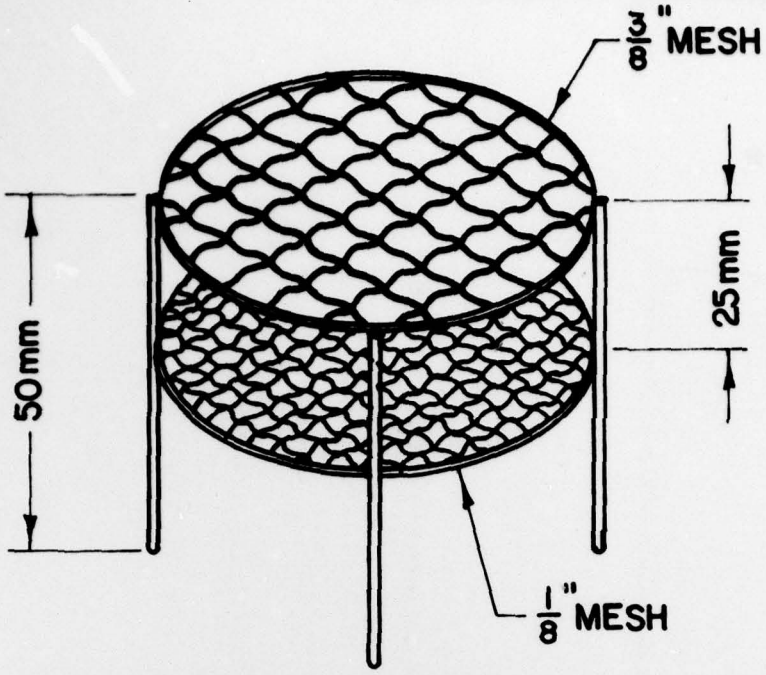

FIG. 2: STAINLESS STEEL SCREEN ASSEMBLY

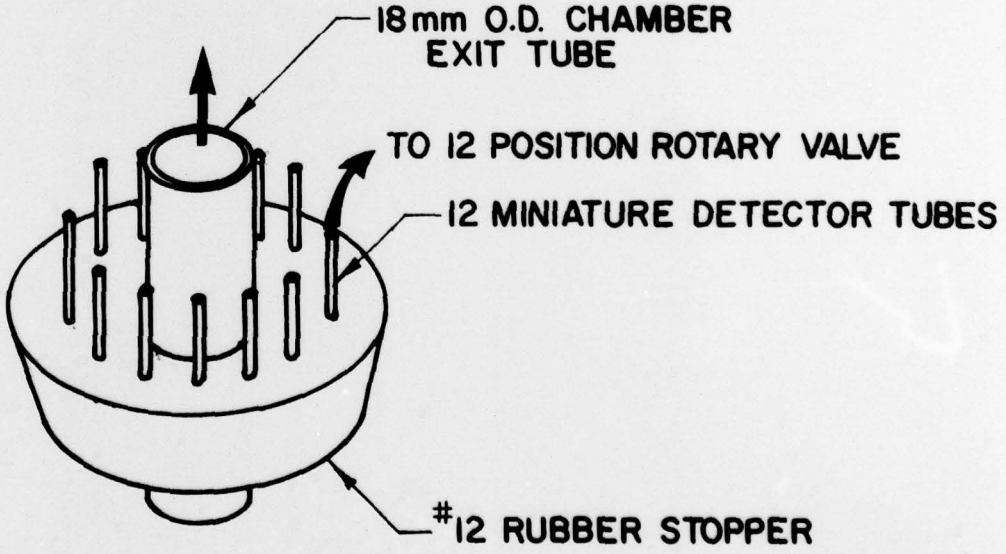

FIG. 3: MINIATURE DETECTOR TUBES
AND CHAMBER EXIT TUBE

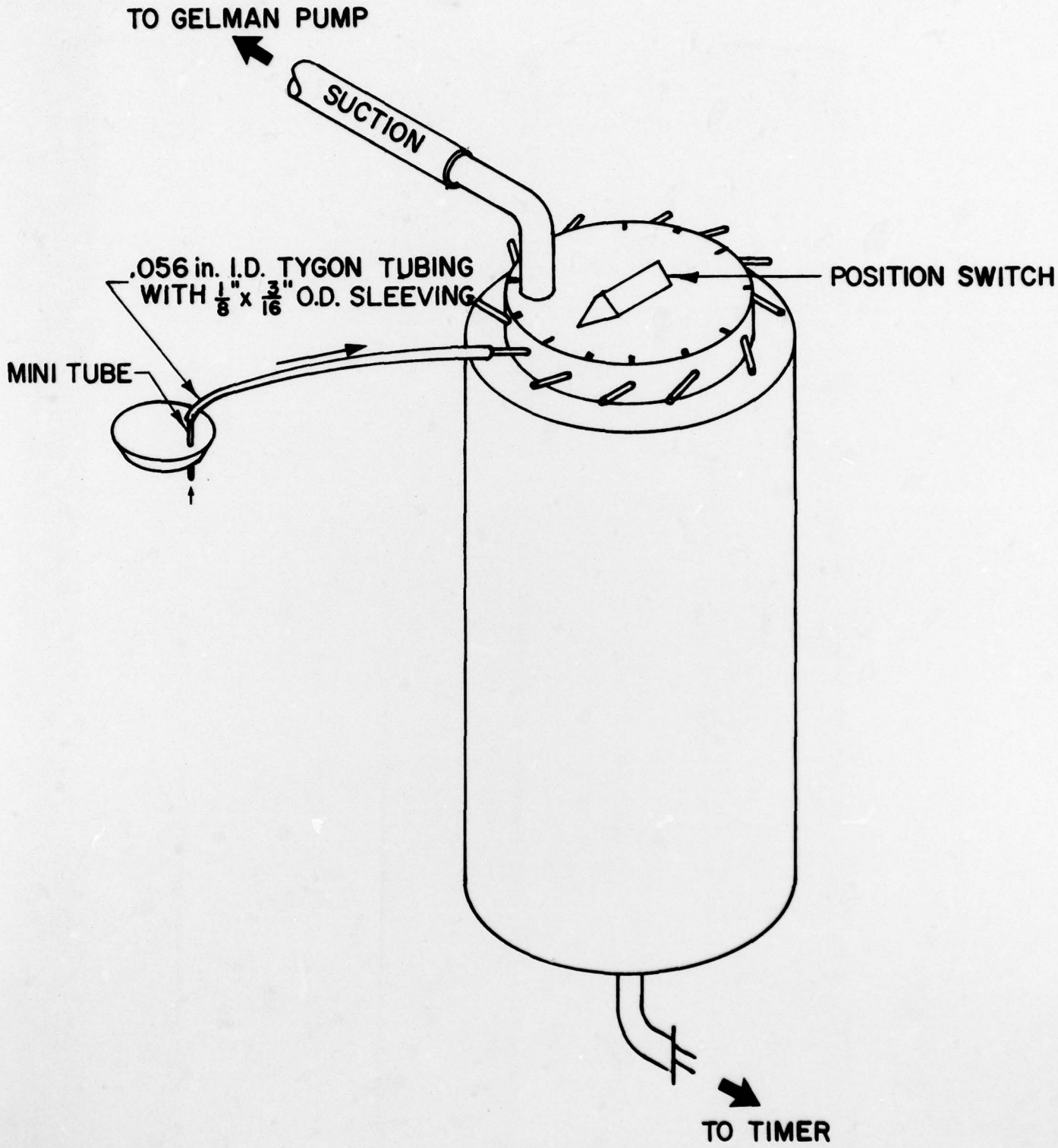

FIG. 4: ROTARY VALVE ASSEMBLY

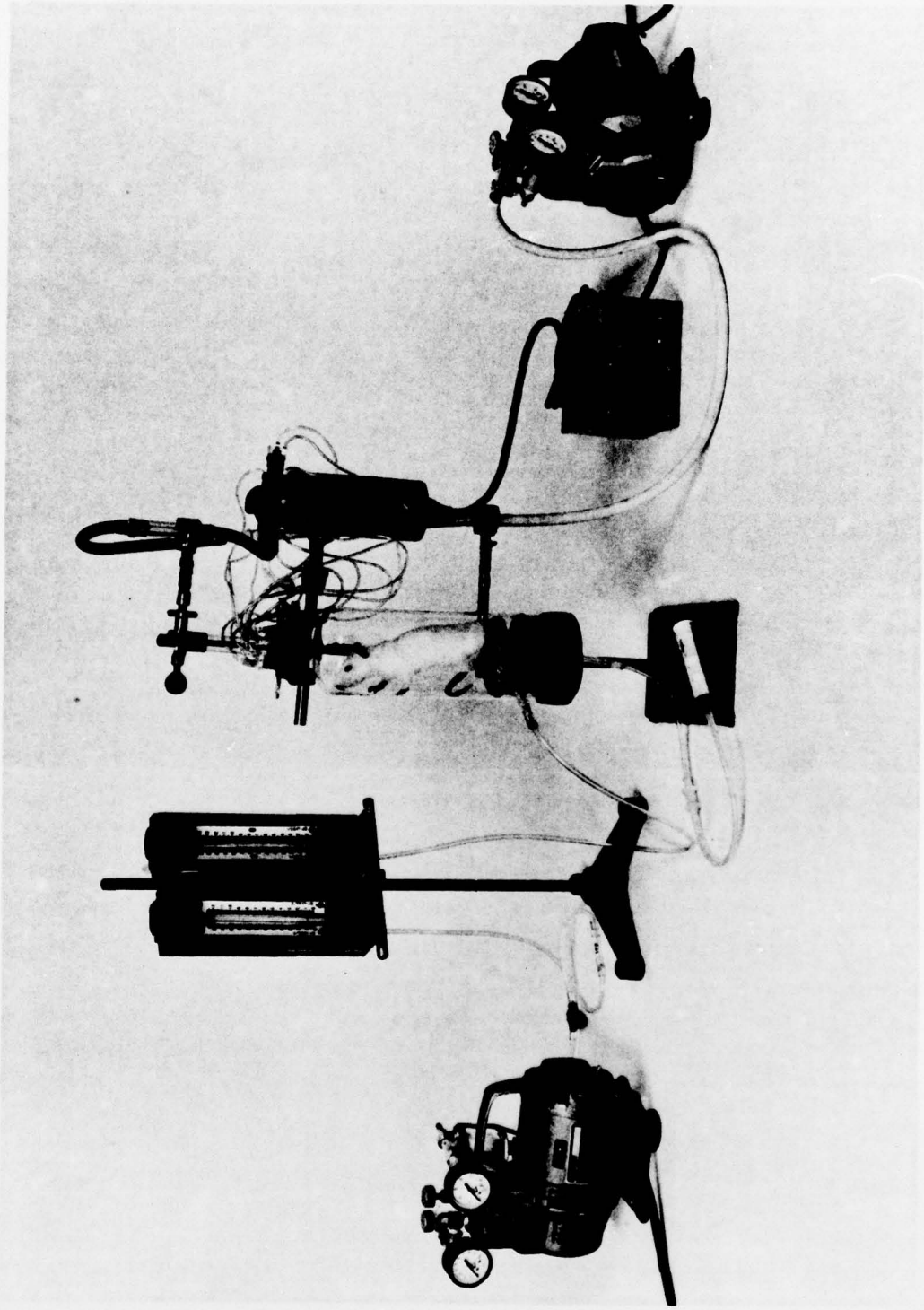

FIG. 5: METABOLIC CHAMBER WITH ANCILLARY EQUIPMENT

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